REVIEW

Large Arf1 guanine nucleotide exchange factors: evolution, domain structure, and roles in membrane trafficking and human disease

Quynh Trang Bui · Marie-Pierre Golinelli-Cohen · Catherine L. Jackson

Abstract The Sec7 domain ADP-ribosylation factor (Arf) guanine nucleotide exchange factors (GEFs) are found in all eukaryotes, and are involved in membrane remodeling processes throughout the cell. This review is focused on members of the GBF/Gea and BIG/Sec7 subfamilies of Arf GEFs, all of which use the class I Arf proteins (Arf1-3) as substrates, and play a fundamental role in trafficking in the endoplasmic reticulum (ER)—Golgi and endosomal membrane systems. Members of the GBF/Gea and BIG/Sec7 subfamilies are large proteins on the order of 200 kDa, and they possess multiple homology domains. Phylogenetic analyses indicate that both of these subfamilies of Arf GEFs have members in at least five out of the six eukaryotic supergroups, and hence were likely present very early in eukaryotic evolution. The homology domains of the large Arf1 GEFs play important functional roles, and are involved in interactions with numerous protein partners. The large Arf1 GEFs have been implicated in several human diseases. They are crucial host factors for the replication of several viral pathogens, including poliovirus, coxsackievirus, mouse hepatitis coronavirus, and hepatitis C virus. Mutations in the BIG2 Arf1 GEF have been linked to autosomal recessive periventricular heterotopia, a disorder of neuronal migration that leads to severe malformation of the cerebral cortex. Understanding the roles of the Arf1 GEFs in membrane dynamics is crucial to a full understanding of trafficking in the secretory and endosomal pathways, which in turn will provide essential insights into human diseases that arise from misregulation of these pathways.

Keywords Guanine nucleotide exchange factor · GBF1 · BIG1 · BIG2 · ADP-ribosylation factor · Small G protein

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Introduction

The distinguishing feature of eukaryotic cells is their internal membrane organization. Membrane-bound organelles such as the endoplasmic reticulum (ER), the Golgi apparatus, and endosomes, ensure that specialized functions are carried out in the appropriate delimited environment. Each organelle possesses a characteristic lipid and protein composition, but is highly dynamic and linked to other organelles via trafficking pathways. There are two major membrane trafficking systems in eukaryotes, the secretory and the endocytic systems. The secretory pathway transports secreted and membrane proteins synthesized in the ER to their final destination (plasma membrane (PM), cell exterior or intracellular organelle). Endocytosis pathways transport material from the cell exterior to intracellular organelles such as the lysosome (or vacuole in yeasts).

The Golgi apparatus is a structurally complex and highly dynamic organelle that is found at the crossroads of the secretory and endosomal membrane trafficking pathways.

The mechanisms that generate and maintain Golgi structure are not well understood. However, activation of the ADP-ribosylation factor 1 (Arf1) small G protein is absolutely required to maintain Golgi structure and function in eukaryotic cells, since inhibiting Arf1 activation by drugs or mutations causes complete disassembly of the Golgi apparatus and a complete block in trafficking pathways through the Golgi (Klausner et al. 1992; Jackson 2009). Membrane trafficking in eukaryotic cells is mediated by transport vesicles that bud from a donor compartment, then are targeted to and fuse with an acceptor compartment (Bonifacino and Glick 2004; Behnia and Munro 2005). Arf1 is required for vesicle budding, where its activation results in recruitment of effectors such as coat complexes to membranes. Coat complexes deform membranes and concentrate cargo into a membrane domain to form a vesicle that carries the cargo from the donor to acceptor compartment. Targeting of a vesicle to its correct acceptor compartment membrane requires tethering molecules that link the vesicle and target membranes at a distance. A series of steps ensues that result in engagement of SNARE proteins, which mediate membrane fusion (Bonifacino and Glick 2004). The proteins involved in vesicle budding, cargo sorting, tethering, and fusion are highly conserved in evolution (Behnia and Munro 2005).

Recent advances in genome sequencing efforts have allowed definition of six major supergroups of eukaryotes (Adl et al. 2005). Interestingly, phylogenetic studies have indicated that the Last Common Eukaryotic Ancestor (the LCEA) possessed a complex endomembrane system and that many of the major families of proteins involved in trafficking arose prior to the divergence of the eukaryotic supergroups (Jekely 2003; Dacks and Field 2007; Gurkan et al. 2007; Kloepper et al. 2007; Pereira-Leal 2008). The LCEA probably already had small G proteins including primordial Arf and Rab proteins, coat complexes, membrane tethering complexes, and SNAREs (Gurkan et al. 2007; Koumandou et al. 2007; Kloepper et al. 2008; Pereira-Leal 2008; Dacks et al. 2009).

There are nine subfamilies of Sec7 domain Arf guanine nucleotide exchange factors (GEFs) in eukaryotes (Cox et al. 2004). In this review, we will focus on two of these subfamilies, the GBF/Gea and BIG/Sec7 GEFs. Other well-studied subfamilies of Arf GEFs include the ARNO/cytohesin, SYT1, SYT2, EFA6, and BRAG subfamilies, which are in general smaller proteins, and which function primarily in endosomal–PM trafficking pathways (Shin and Nakayama 2004; Casanova 2007; Gillingham and Munro 2007). Of the nine subfamilies of Arf GEFs, only those of the GBF/Gea, BIG/Sec7, ARNO/cytohesin, and RalF subfamilies have significant activity on the class I Arfs, Arf1–Arf3. RalF is the founding member of a family of bacterial Arf1 GEFs, first found in *Legionella pneumophila* and *Rickettsia prowazekii* (Nagai et al. 2002; Amor et al. 2005).

These proteins do not arise from the bacteria themselves, but were incorporated into these bacterial genomes through horizontal transfer from their eukaryotic host (Nagai et al. 2002). We will not discuss this special subfamily of Arf1 GEFs in this review. Among the eukaryotic Arf1 GEFs, there has been some controversy as to whether ARNO/cytohesin GEFs actually use class I Arfs as substrates in vivo, as they localize primarily to the endosomal–PM system where Arf6 functions. However, a key paper from the Donaldson laboratory has provided new insights into this issue through identification of a GTPase cascade involving both Arf1 and Arf6 in cells (Cohen et al. 2007). Cohen et al. demonstrated that interaction of Arf6-GTP with the PH domain of ARNO is essential for its recruitment to the PM, where it then activates Arf1. Structural and biochemical analyses showing release of an autoinhibitory interaction within ARNO by Arf6-GTP provide further molecular insight into this regulatory cascade (DiNitto et al. 2007). Hence, although the ARNO/cytohesin proteins are bona fide Arf1 GEFs, we will focus this review on the GBF/Gea and BIG/Sec7 Arf1 GEFs, which function in Golgi and endosomal membrane trafficking pathways.

The GBF/Gea and BIG/Sec7 subfamilies are related, with members sharing sequence similarity principally in five homology domains (Mouratou et al. 2005). The majority of these Arf1 GEFs are high molecular weight proteins, on the order of 200 kDa, and for this reason they have been referred to as the large Arf GEFs. The GBF/Gea and BIG/Sec7 Arf1 GEFs function in internal membrane systems such as the Golgi apparatus, the *trans*-Golgi network (TGN) and endosomal pathways (Zhao et al. 2002; Shin and Nakayama 2004; Park et al. 2005; Casanova 2007; Gillingham and Munro 2007).

Although the GBF/Gea and BIG/Sec7 Arf1 GEFs share a common domain structure, they clearly form two distinct subfamilies (Cox et al. 2004; Mouratou et al. 2005). Members of each subfamily have different localizations and functions, with GBF/Gea GEFs in general functioning in the ER-early Golgi membrane system and BIG/Sec7 GEFs functioning in the late-Golgi and endosomal membrane systems. Mammalian GBF1 localizes primarily to the intermediate compartment between the ER and the Golgi (ERGIC) and to *cis*-Golgi membranes (Kawamoto et al. 2002; Zhao et al. 2002; Garcia-Mata et al. 2003), and yeast Gea1p and Gea2p localize to the *cis*-Golgi (Chantalat et al. 2003). In plants, all the Arf GEFs fall into the GBF/Gea and BIG/Sec7 subfamilies, so the situation is more complicated. Like its yeast and mammalian counterparts, the *A. thaliana* Arf1 GEF GNL1 localizes to and functions at the *cis*-Golgi, but another member of the GBF/Gea subfamily, GNOM, localizes to and functions in the endosomal membrane system, although it still retains a capacity to function in the early Golgi (Richter et al. 2007). To compensate for the

lack of endosomal–PM Arf GEFs, plants have thus diversified the functions of the GBF/Gea subfamily members, and possibly those of the BIG/Sec7 subfamily as well. In mammals and yeast, the BIG/Sec7 proteins localize to and function at the *trans*-Golgi, the TGN and endosomes (Franzusoff et al. 1991; Shinotsuka et al. 2002a; Shinotsuka et al. 2002b; Zhao et al. 2002; Charych et al. 2004; Shin et al. 2004).

In this review, we will discuss the evolution of these Arf1 GEFs, and present an analysis of conserved domains that are common to both subfamilies as well as those that are specific to either the GBF/Gea or BIG/Sec7 proteins. We will describe the interacting partners of these Arf1 GEFs, indicating which of the homology regions of these proteins are involved in each interaction. Finally, we will describe studies that have implicated the Arf1 GEFs in human disease, including recent studies describing specific inhibitors of members of the GBF/Gea subfamily of Arf1 GEFs.

Phylogenetic analysis of the Arf1 GEFs

The number of complete genome sequences has increased dramatically in the past few years, and now are available in public databanks for a wide variety of both prokaryotes and eukaryotes. Analyses of these genome sequences have indicated that there are six major supergroups of eukaryotes that diverged from the LCEA (Adl et al. 2005). These supergroups are the Excavata, the Chromalveolata, the Archaeplastidia, the Opisthokonta, the Amoebozoa, and the Rhizaria. Fungi, including the well-studied model system *Saccharomyces cerevisiae* and mammals, are part of the same supergroup, the Opisthokonta. Complete genome sequences from members of five of the six eukaryotic supergroups are available, with the Rhizaria being the one exception. We searched the complete genome sequences of representative members of these five supergroups for members of the two subfamilies of large Arf1 GEFs, and found members of both the GBF/Gea and the BIG/Sec7 proteins (Tables 1, 2). Humans have two BIG/Sec7 proteins, called BIG1 and BIG2, and only one GBF/Gea subfamily member, GBF1. Arabidopsis, which like other plant species of the Archaeplastida, have only GBF/Gea and BIG/Sec7 Arf1 GEF subfamilies and no others, have five BIGs and three GBFs. *Paramecium tetraurelia*, a protozoan that belongs to the Chromalveolata supergroup, has nine BIG/Sec7 members, but no GBF/Gea sequences. The model organism *Saccharomyces cerevisiae* has one member of the BIG/Sec7 subfamily, Sec7p, and two members of the GBF/Gea subfamily, Gea1p and Gea2p (likely due to a recent genome duplication event). The human pathogen *Cryptosporidium parvum*, a member of the Apicomplexa, has no BIG/Sec7 proteins, and only one GBF/Gea sequence. The *C. parvum*

GBF protein shares a high level of homology with plant GBF proteins such as the *Arabidopsis thaliana* GNOM protein. The Apicomplexa are characterized by the apicoplast, an organelle which is thought to be the remnant of an endosymbiotic algal cell (Abrahamsen et al. 2004; Huang et al. 2004). It is likely that the *C. parvum* GBF gene, like other plant-like genes in this organism, was transferred from the primordial endosymbiosed algal cell to the nucleus of the Apicomplexan ancestor that engulfed it (Huang et al. 2004). The closely related *Cryptosporidium hominis* species has a very divergent Arf GEF that appears nevertheless to be a member of the GBF/Gea subfamily (Xu et al. 2004).

Phylogenetic analysis of the BIG/Sec7 and GBF/Gea subfamilies of Arf GEFs using members from five eukaryotic supergroups indicates a clear separation of these two subfamilies (Fig. 1). The entire Arf GEF sequences were used for the phylogenetic analysis, not just the Sec7 domains as in previous studies (Cox et al. 2004; Mouratou et al. 2005). Using three different programs, the same tree topology was obtained, with high confidence levels for a separation of the BIG/Sec7 and GBF/Gea sequences into two different clades (Fig. 1). Hence the presence of two subfamilies of large Arf GEFs is ancient, and it is likely that both GBF/Gea and BIG/Sec7 proteins existed within the LCEA before the separation of the different eukaryotic lineages. The nine protozoan *Paramecium tetraurelia* Arf GEFs, including the five denoted GGG1-5, clearly fall into the BIG/Sec7 subfamily (Fig. 1).

Homology domains of the GBF/Gea and BIG/Sec7 Arf1 GEFs

As described previously, the large Arf1 GEFs have five major sequence homology domains that are common to members of both the GBF/Gea and the BIG/Sec7 subfamilies (Mouratou et al. 2005). These domains are the DCB and the HUS domains upstream of the catalytic Sec7 domain, and HDS1, HDS2, and HDS3 that lie downstream of the Sec7 domain (Fig. 2). This analysis was based on sequence data from organisms found primarily within two eukaryotic supergroups. When representative members of five of the six eukaryotic supergroups are analyzed, the five common homology domains are present in the Arf1 GEFs of most of the organisms examined, in both GBF/Gea and BIG/Sec7 subfamily members (Figs. 3, 4, 5, 6, 7, 8). However, HDS2 and HDS3 are the least conserved domains, particularly for the GBF/Gea proteins, and we were unable to find evidence for these domains in the GBF protein of the Chromalveolata supergroup member *C. parvum*.

Table 1 Sequences used for the phylogenetic analysis and multiple alignments

Super-groups	Groups	Species	Protein name	Accession number	Size in amino acids
Opisthokonta	Mammals	*Canis familiaris*	BIG1 Canf	XP_535095.2	1,849
			GBF1 Canf	XP_850976.1	1,872
		Equus caballus	BIG1 Equc	XP_001494609.1	1,840
			GBF1 Equc	XP_001499167.1	1,858
		Homo sapiens	BIG1 Homs[a]	Q9Y6D6.2	1,849
			BIG2 Homs[a]	Q9Y6D5.3	1,785
			GBF1 Homs[a]	Q92538.2	1,859
		Monodelphis domestica	BIG1 Mond	XP_001368081.1	1,849
			GBF1 Mond	XP_001369356.1	1,861
		Mus musculus	BIG1 Musm	XP_001473216	1,846
			GBF1 Musm	NP_849261.2	1,861
		Pan troglodytes	BIG1 Pant	XP_519797.2	1,849
			GBF1 Pant	XP_521592.2	1,859
		Rattus norvegicus	BIG1 Ratn[a]	EDM11544.1	1,846
			GBF1 Ratn[a]	XP_001066196.1	1,862
	Invertebratesss	*Anopheles gambiae*	BIG Anog[a]	XP_319652.3	1,662
			GBF Anog[a]	XP_321598.4	2,000
		Drosophila melanogaster	BIG Drom[a]	NP_609675.2	1,653
			GBF Drom[a]	NP_610761.2	1,983
		Nasonia vitripennis	BIG Nasv	XP_001605970.1	1,818
			GBF Nasv	XP_001601088.1	1,767
		Tribolium castaneum	BIG Tric	XP_972785.1	1,663
			GBF Tric	XP_967092.1	1,742
	Fungi	*Candida glabrata*	SEC7 Cang	XP_445724.1	1,825
			GEA Cang	XP_447385.1	1,454
		Kluyveromyces lactis	GEA Klul[a]	XP_454979.1	1,387
			SEC7 Klul[a]	XP_453828.1	1,848
		Saccharomyces cerevisiae	GEA1 Sacc[a]	P47102.1	1,408
			GEA2 Sacc[a]	P39993	1,459
			SEC7 Sacc[a]	P11075.2	2,009
		Schizosaccharomyces pombe	SEC71 Schp	Q9UT02.1	1,811
			SEC72 Schp	Q9P7V5.1	1,822
			GEA Schp	CAB75411.1	1,462
Amoebozoa	Mycetozoa	*Dictyostelium discoideum*	BIG Dicd[a]	XP_635808.1	1,886
			GBF Dicd[a]	XP_635561.1	1,748
Archaeplastida	Flowering	*Arabidopsis thaliana*	BIG1 Arat	AAF76474.1	1,750
			BIG2 Arat	NP_191645.1	1,793
			BIG3 Arat	NP_195533.1	1,698
			BIG4 Arat[a]	O65490	1,711
			GNL1 Arat	Q9FLY5	1,443
			GNL2 Arat	NP_197462.1	1,375
			GNOM Arat[a]	Q42510.1	1,451
		Oryza sativa	BIG Orys[a]	AAM00191.1	1,789
			GBF3 Orys[a]	EAY91294.1	1,456
		Vitis vinifera	BIG Vitv	CAO24490.1	1,653
			GBF3 Vitv	CAN61434.1	1,433

Table 1 continued

Super-groups	Groups	Species	Protein name	Accession number	Size in amino acids
	Algae	*Chlamydomonas reinhardtii*	BIG Chlr[a]	XP_001694943.1	2,150
			GBF Chlr[a]	XP_001700166.1	1,490
		Ostreococcus tauri	BIG Ostt[a]	CAL51840.1	1,743
Chromalveolata	Alveolata	*Tetrahymena thermophila*	BIG Tett[a]	XP_001031228.2	1,842
		Cryptosporidium parvum	GBF Cryp[a]	XP_625855.1	1,800
Excavata	Protist	*Trichomonas vaginalis*	BIG Triv[a]	XP_001327074.1	1,305
			GBF Triv[a]	XP_001316830.1	1,204

[a] Indicates sequences used for the alignments in Figs. 3, 4, 5, 6, 7, and 8

Table 2 Additional sequences used only for phylogenetic analysis

GEF subfamily	Species	Protein name	Accession number	Size in amino acids
GBF/Gea	*Canis lupus familiaris*	GBF1 Canf	XP_850976.1	1,872
	Danio rerio	GBF1 Danr	XP_694714.3	2,059
	Gallus gallus	GBF1 Galg	XP_421632.2	1,852
	Caenorhabditis elegans	GBF Caee	NP_499522.2	2,005
	Strongylocentrotus purpuratus	GBF Strp	XP_001193947.1	1,447
	Hydra magnipapillata	GBF Hydm	XP_002167603.1	1,705
	Ciona intestinalis	GBF Cioi	XP_002128867.1	1,842
	Neurospora crassa	GBF Neuc	XP_962693.2	1,588
	Ustilago maydis	GBF Ustm	XP_757309.11738	1,738
	Monosiga brevicollis	GBF Monb	XP_001742906.1	1,541
	Coprinopsis cinerea okayama	GBF Copo	XP_001833466.1	1,499
BIG/Sec7	*Rattus norvegicus*	BIG2 Ratn	NP_851597	1,791
	Canis lupus familiaris	BIG1 Canf	XP_859391.1	1,859
	Danio rerio	BIG1 Danr	XP_001923315.1	1,833
	Gallus gallus	BIG1 Galg	XP_418283.2	1,537
	Caenorhabditis elegans	BIG Caee	NP_001129800.1	1,628
	Strongylocentrotus purpuratus	BIG Strp	XP_791125.2	1,396
	Ciona intestinalis	BIG Cioi	XP_002123264.1	1,689
	Neurospora crassa	BIG Neuc	XP_962785.2	1,948
	Ustilago maydis	BIG Ustm	XP_760498.1	2,038
	Monosiga brevicollis	BIG Monb	XP_001748607.1	1,786
	Coprinopsis cinerea okayama	BIG Copo	XP_001837141.1	1,910
	Paramecium tetraurelia	GGG1 Part	XP_001428865	1,615
		GGG2 Part	XP_001437414	1,615
		GGG3 Part	XP_001429046	1,598
		GGG4 Part	XP_001431359	1,599
		GGG5 Part	XP_001450751	1,473

Because the GBF/Gea and the BIG/Sec7 proteins have distinct localizations and functions, it is pertinent to ask the question of whether there exist homology regions specific to each of the two subfamilies of large Arf1 GEFs. Examination of homology regions both upstream and downstream of the Sec7 domain indicated that among the representatives of all five eukaryotic supergroups, only one domain, specific to the BIG/Sec7 subfamily, lies outside of the common domains (Fig. 10). All other regions of high sequence conservation specific to either the GBF/Gea or the BIG/Sec7 subfamilies
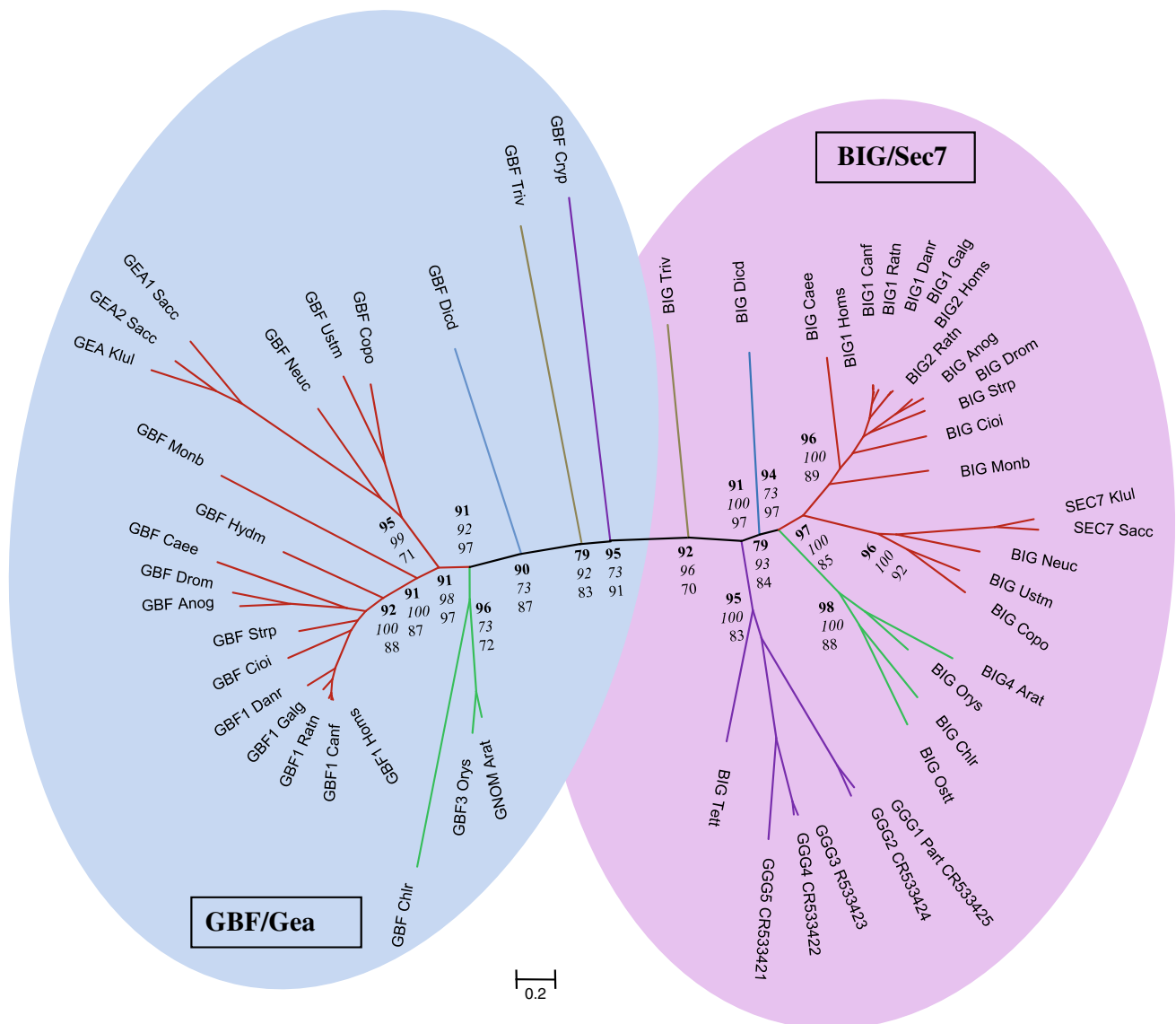
Fig. 1 Unrooted phylogenetic tree of the BIG/Sec7 and GBF/Gea Arf GEFs using the full-length sequence of each protein. Three separate analyses were performed using the programs MrBayes (Bayesian inference), PAUP (maximum parsimony), and PhyML (maximum likelihood). Bootstrap percentages are shown for each, with results using MrBayes in *bold*, PAUP in *italics*, and a PhyML in *normal text*.

The sequences used for the analysis are shown in Tables 1 and 2. Branches of the tree are color coded to indicate the eukaryotic supergroup that each sequence belongs to: Opisthokonta (*red*), Archaeplastidia (*green*), Amoebozoa (*blue*), Chromalveolata (*violet*), and Excavata (*beige*)

that were present among members of all the eukaryotic supergroups we examined were within the common homology regions DCB, HUS, HDS1, HDS2, and HDS3. Hence it appears that there is a highly conserved function and/or structure of the large Arf1 GEFs that is preserved in both the subfamilies, and specific functions are superposed on this common structural organization. Overall, there is a higher level of sequence homology between the BIG/Sec7 subfamily members than among GBF/Gea sequences.

Outside of the catalytic Sec7 domain, which is the most highly conserved domain of the Arf1 GEFs, the N-terminal domains DCB and HUS have the next highest levels of homology, particularly among GBF/Gea family members. As described previously (Ramaen et al. 2007), the DCB domain was redefined to include residues upstream of the originally defined domain (Mouratou et al. 2005) (Fig. 3). The most highly conserved region outside of the catalytic Sec7 domain is the "HUS box," a region of seven amino
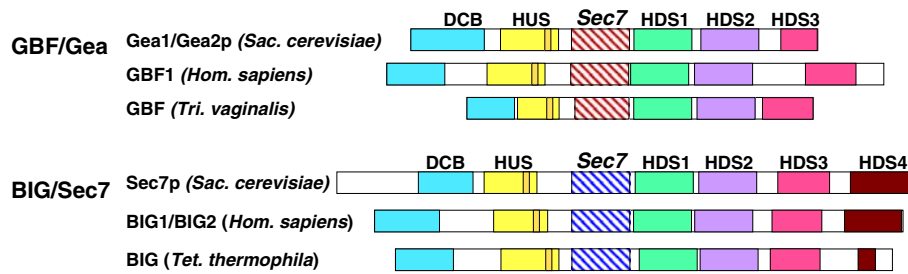
Fig. 2 Domain structure of the BIG/Sec7 and GBF/Gea Arf GEFs. Positions of the homology domains are shown for representative sequences of the Opisthokonta supergroup (*Saccharomyces cerevisiae*, *Homo sapiens*), and of the Excavata and Chromalveolata supergroups (*Trichomonas vaginalis*, *Tetrahymena thermophila*); positions of domains and sizes of proteins are drawn to scale. The only homology domain unique to one subfamily is the BIG/Sec7 HDS4 sequence; only a portion of the region shown in Fig. 10 is conserved in the Chromalveolata members that we analyzed (amino acid residues 1,600–1,665 of the *Tetrahymena thermophila* BIG protein, corresponding to amino acids 1,675–1,740 of human BIG1). The *orange box* within the HUS domain represents the highly conserved HUS box, sequence Y/F Φ N Y/F D C D/E/N (Φ: hydrophobic)

acids near the end of the HUS domain (Fig. 4) (Mouratou et al. 2005; Park et al. 2005). Including a wide range of the eukaryotic sequences confirms the high level of homology of this region, which has the consensus sequence Y/F Φ N Y/F **D C** D/E/N (where Φ stands for hydrophobic). The highly conserved aspartic acid residue within this motif is invariant in all but one of the sequences examined (Fig. 4). In vivo, this residue plays an important role in trafficking and in GBF/Gea function in both mammalian cells and in yeast (Park et al. 2005; Ramaen et al. 2007; Deng et al. 2009). On either side of this motif, there are residues conserved within each subfamily of the Arf1 GEFs, specific either for GBF/Gea or BIG/Sec7 proteins (Fig. 4).

In the catalytic Sec7 domain, the loop between helices α6 and α7 in yeast, plant, and mammalian large Arf1 GEFs has the invariant sequence FRLPGE, where the final residue is the glutamic acid finger that is essential for exchange activity (Goldberg 1998; Beraud-Dufour et al. 1999). When members of other eukaryotic families are included, only residues F and GE of this motif are invariant (Fig. 5). Significantly, the catalytic glutamic acid residue is invariant even in this wide range of eukaryotic organisms. Within the Sec7 domain itself, there are several residues that are highly conserved within each subfamily (Fig. 5). Again, there are more conserved residues among BIG/Sec7 subfamily members than among GBF/Gea sequences.

In the C-terminal homology regions HDS1, HDS2, and HDS3, the BIG/Sec7 sequences had a significantly higher level of homology than the GBF/Gea sequences (Figs. 6, 7, 8). Both HDS1 and HDS2 are more highly conserved among the BIGs than among the GBFs (Figs. 6, 7), with the second half of HDS2 showing a particularly high number of conserved residues among BIG/Sec7 proteins of diverse eukaryotic origin (Fig. 7). Indeed, the NCBI conserved domain database has identified a portion of the BIG/Sec7 HDS2 domain as highly conserved and named it DUF1981. In contrast, there are few if any residues in the second half of HDS2 that are conserved among all the GBF/Gea proteins (Fig. 7).

The HDS3 domain was described previously, but our analysis has indicated that this domain is 50–80 amino acids longer than that proposed by Mouratou et al. (2005), with the additional homology region located at the C-terminal end of the originally defined HDS3 domain (Fig. 8). Including representatives of the five eukaryotic super families indicates that there is only weak homology within this domain (Fig. 8), but when representative members of only fungi, plants, and mammals are included, a significant level of homology is found throughout the HDS3 domain among members of both BIG/Sec7 (not shown) and GBF/Gea subfamilies (Fig. 9).

There is one homology region specific to a subfamily of the large Arf1 GEFs, which we call HDS4, and which is present only among BIG/Sec7 proteins (Fig. 10). No equivalent homology region can be found in the GBF/Gea proteins. We found HDS4 sequences in eukaryotic supergroups other than the Opisthokonta (animals, fungi) and Archaeplastida (plants, algae), but with less homology. Both *Tetrahymena thermophila* and *Paramecium tetraurelia* BIG sequences have a region homologous to amino acids 1,675–1,740 of the human BIG1 HDS4 domain (Fig. 2, data not shown). However, we did not find evidence for an HDS4 domain in the Amoebozoa or Excavata supergroup members *Dictyostelium discoideum* and *Trichomonas vaginalis*, respectively.

Interacting Partners of the large Arf1 GEFs

The highly conserved sequence homology domains of the GBF/Gea and the BIG/Sec7 Arf1 GEFs suggest that they have important, conserved functions in evolution, and are likely to be involved in protein–protein interactions. Indeed, studies from a number of laboratories have
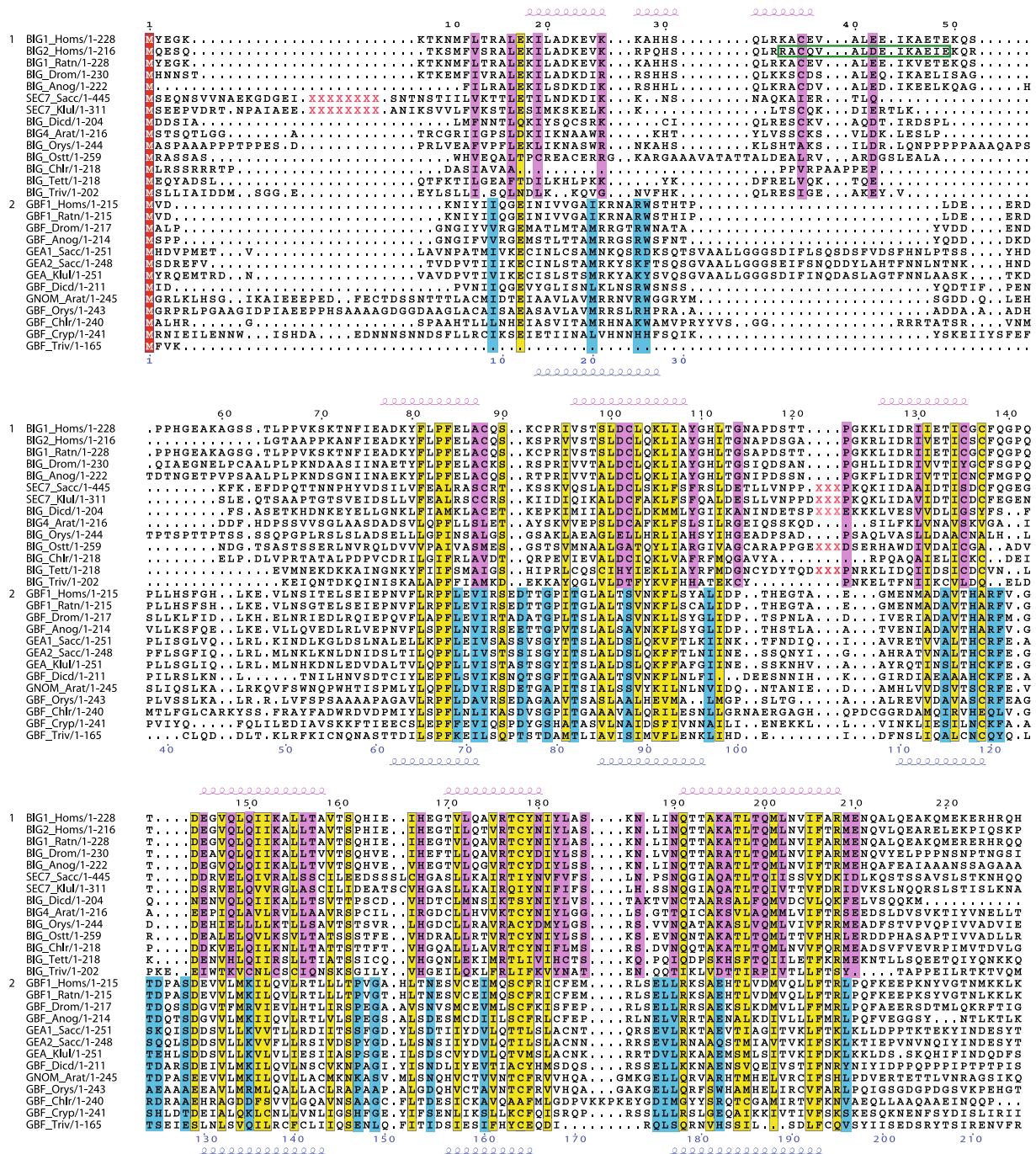
Fig. 3 Conserved residues within the N-terminal region of the BIG/Sec7 and GBF/Gea subfamilies of Arf GEFs containing the DCB domain. Multiple sequence alignment showing conserved residues specific to the BIG/Sec7 subfamily (*pink*), specific to the GBF/Gea subfamily (*blue*), or both subfamilies (*yellow*). Invariant residues in all the sequences are shown in *red*. The most highly conserved portion of this region contains the DCB domain. Secondary structure prediction of alpha-helical regions is shown above alignment in *pink* for BIG/Sec7 sequences, and below alignment in *blue* for GBF/Gea sequences. Deletions in sequences are indicated by *red Xs*, and correspond to: S18-G217, S319-P336 in SEC7_Sacc, R17-M82, S186-P202 in SEC7_Klul, P106-G109 in BIG_Dicd, V120-H154 in BIG_Ostt, V89-N116 in BIG_Chlr, and K101-D113 in BIG_Tett. Protein sequence alignments were created using Clustal W 1.83 (Thompson et al. 1994) and T-coffee (Notredame et al. 2000) with default parameters. The multiple alignments were manually adjusted and edited using BioEdit version 7.0.8 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The GBF/Gea and BIG/Sec7 alignments (done separately) were imported into BioEdit, then these were manually corrected to correspond to the combined alignment. Aligned sequences were displayed with ESPript (Gouet et al. 1999) using the BLOSUM62 matrix with a similarity global score of 0.15 and a difference score between conserved groups of 0.5. Secondary structure predictions on multiple alignments were performed at the Pôle Bioinformatique Lyonnais (http://pbil.univ-lyon1.fr/) and the consensus of three different programs (the PHD, Predator, and GOR IV) is indicated. *Green box* indicates AKAP domain of BIG2

Fig. 4 Conserved residues within the N-terminal region containing the HUS domain. Multiple sequence alignment showing conserved residues specific to the BIG/Sec7 subfamily (*pink*), specific to the GBF/Gea subfamily (*blue*), or both subfamilies (*yellow*). Secondary structure prediction as in Fig. 2. *Green box* indicates AKAP domain of BIG2
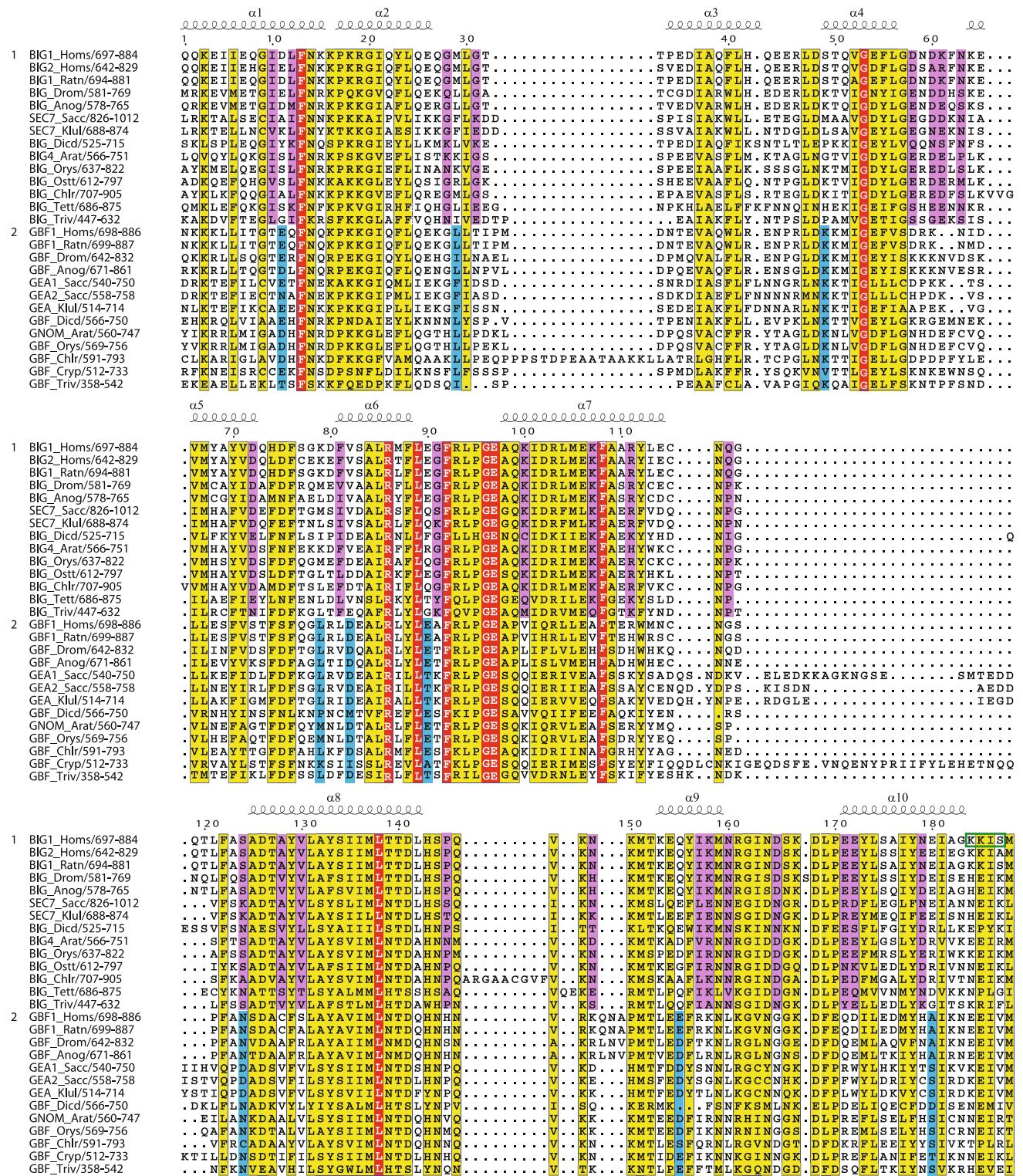
Fig. 5 Conserved residues within the Sec7 domain. Multiple sequence alignment showing conserved residues specific to the BIG/Sec7 subfamily (*pink*), specific to the GBF/Gea subfamily (*blue*), or both subfamilies (*yellow*). Invariant residues in all the sequences are shown in *red*. Alpha-helical regions from crystal structures of Sec7 domains is shown. *Green box* indicates PKA phosphorylation site identified in BIG1
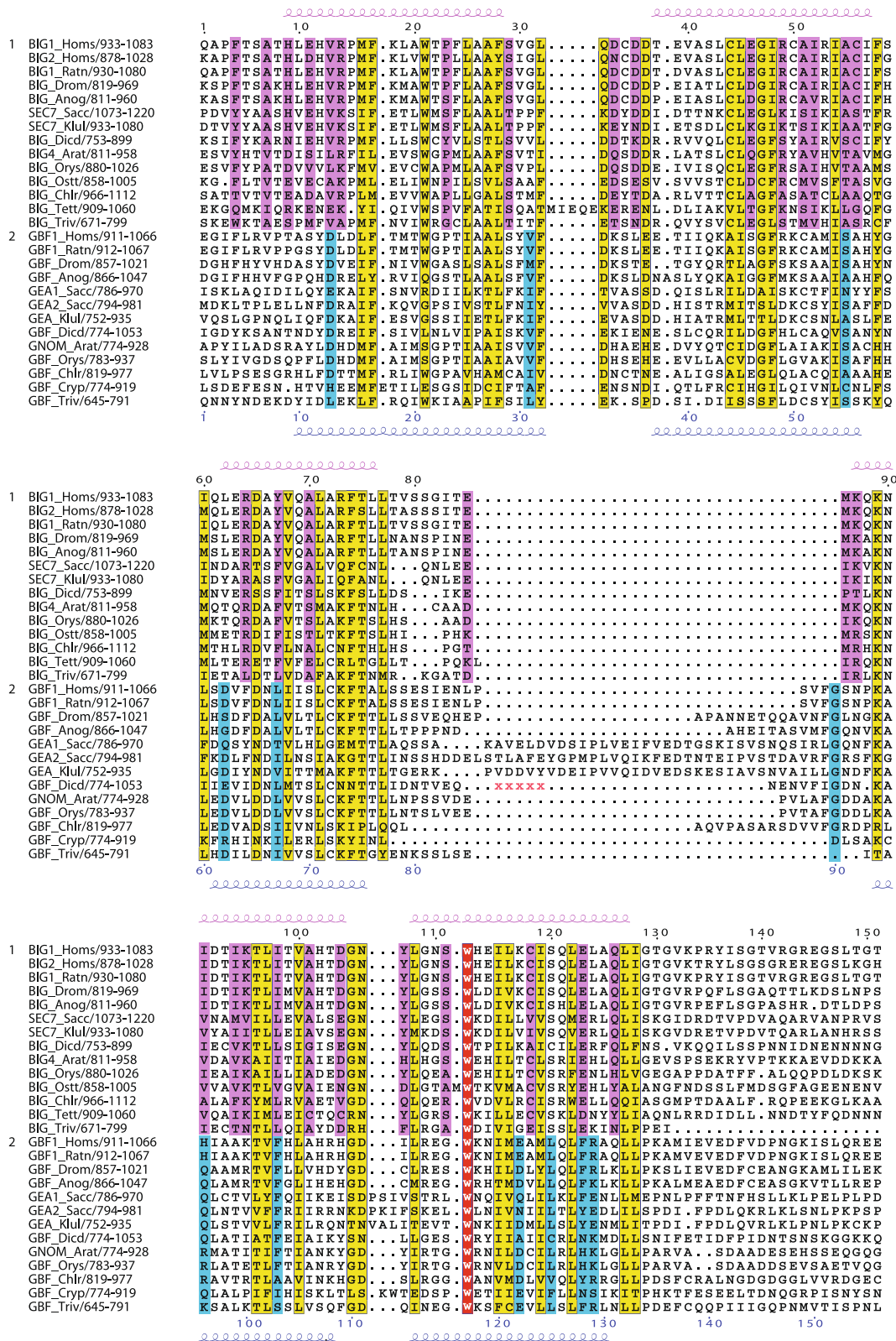
Fig. 6 Conserved residues within the C-terminal region including the HDS1 domain. Multiple sequence alignment showing conserved residues specific to the BIG/Sec7 subfamily (*pink*), specific to the GBF/Gea subfamily (*blue*), or both subfamilies (*yellow*). Invariant residues in all the sequences are shown in *red*. Secondary structure prediction as in Fig. 2. Deletions in sequences are indicated by *red Xs*, and correspond to: Q858-Y982 in GBF_Dicd
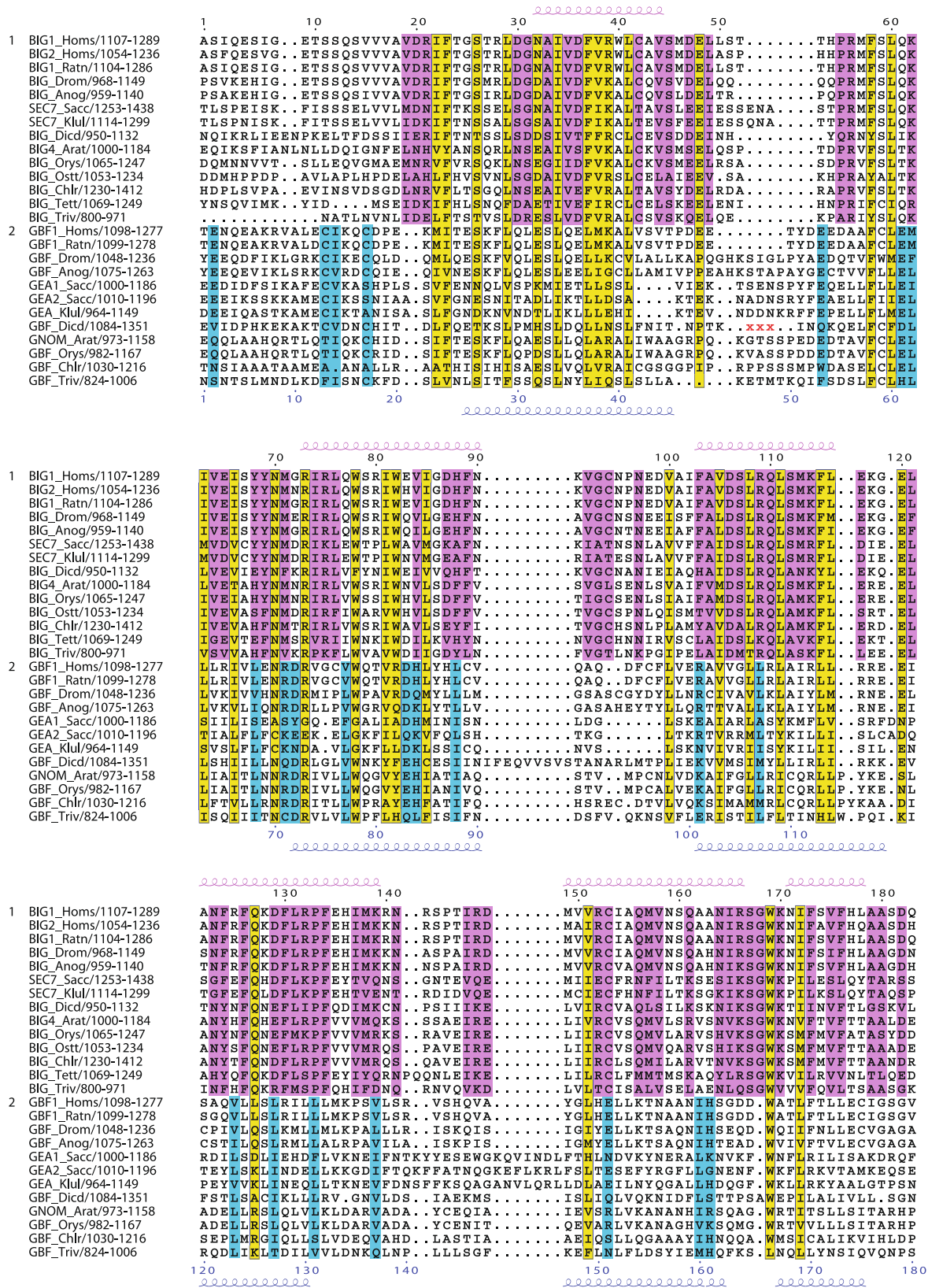
Fig. 7 Conserved residues within the C-terminal region including the HDS2 domain. Multiple sequence alignment showing conserved residues specific to the BIG/Sec7 subfamily (*pink*), specific to the GBF/Gea subfamily (*blue*), or both subfamilies (*yellow*). Secondary structure prediction as in Fig. 2. Deletions in sequences are indicated by *red Xs*, and correspond to: Q1133-Y1211 in GBF_Dicd
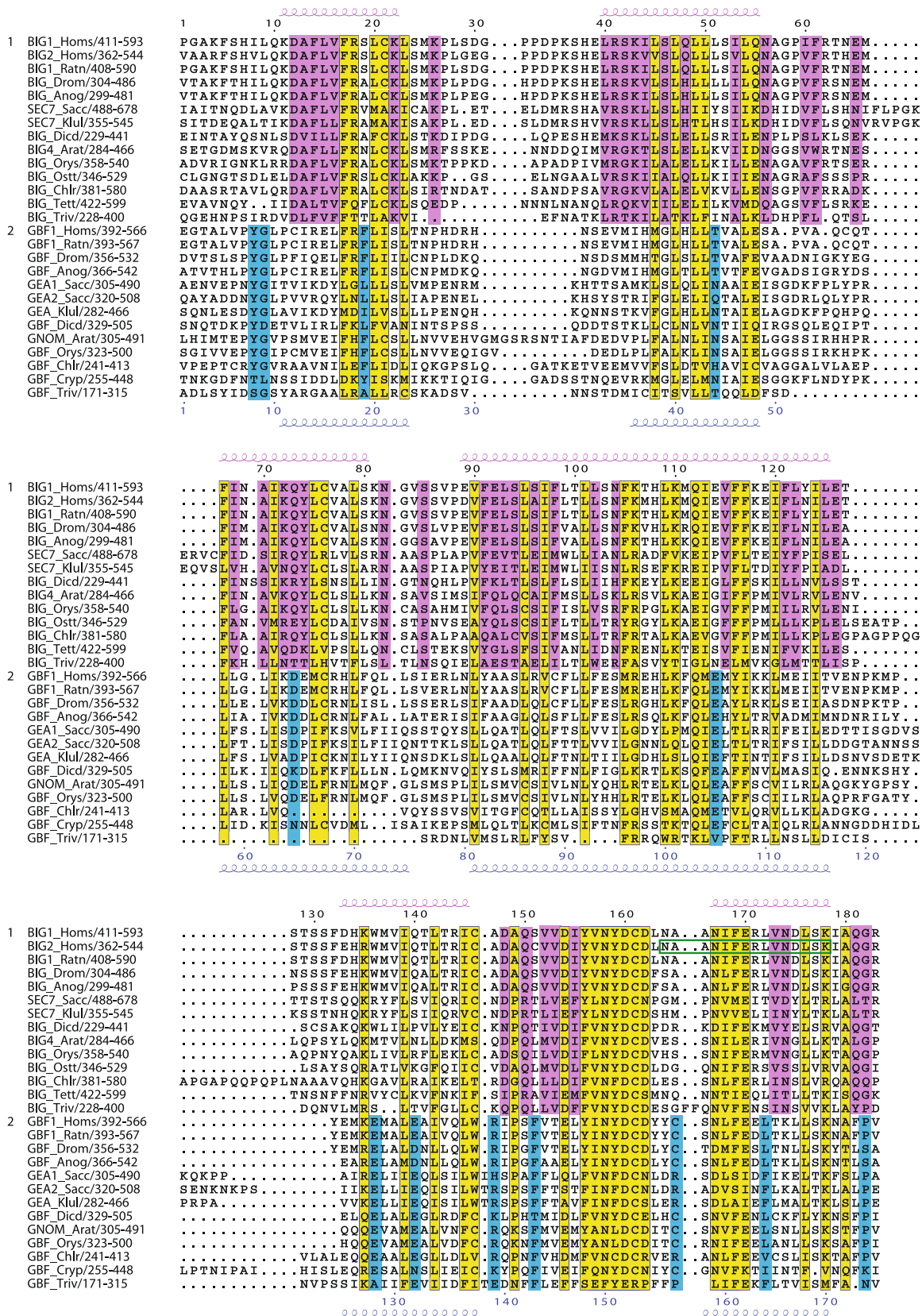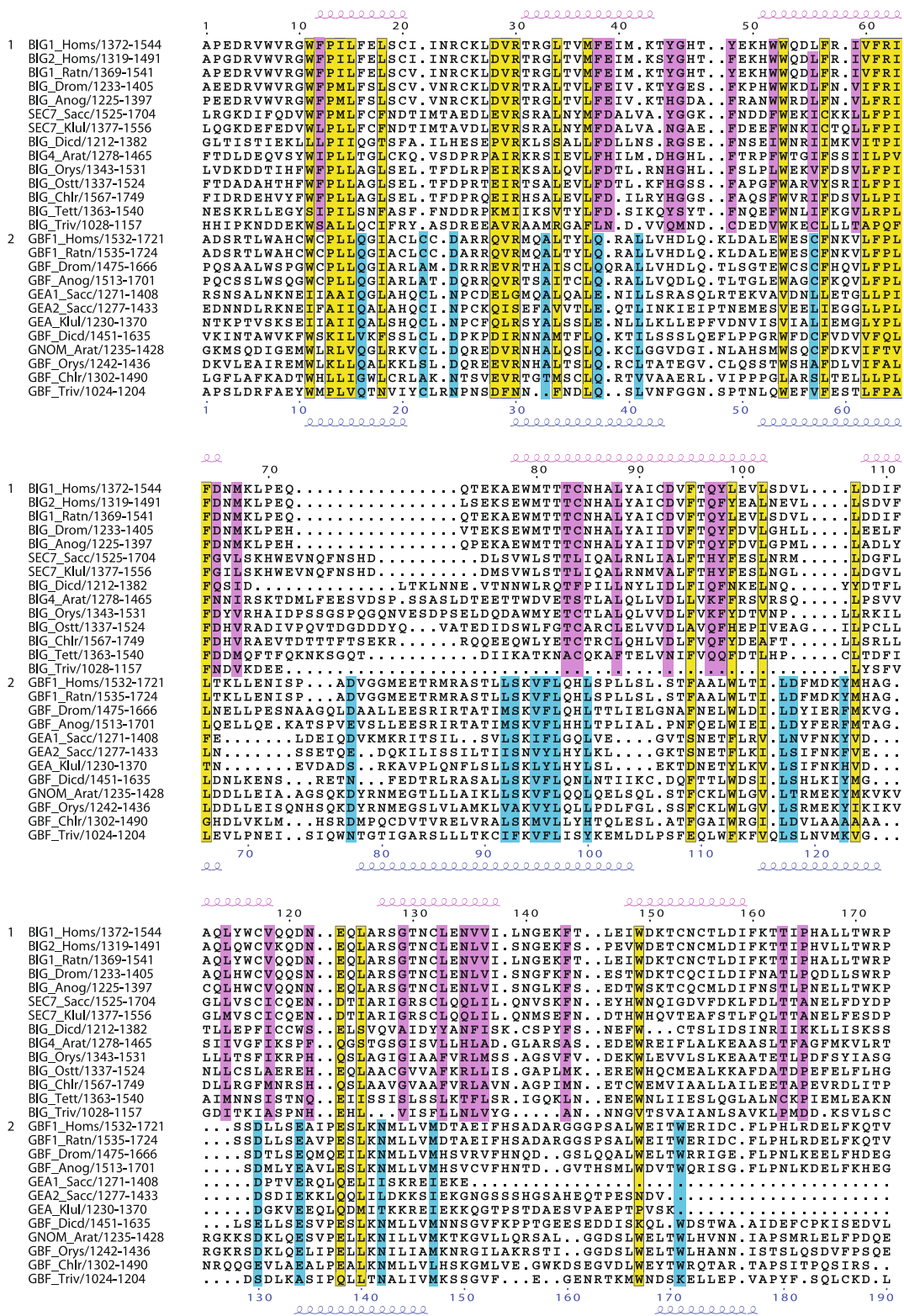
Fig. 8 Conserved residues within the C-terminal region including the HDS3 domain. Multiple sequence alignment showing conserved residues specific to the BIG/Sec7 subfamily (*pink*), specific to the GBF/Gea subfamily (*blue*), or both subfamilies (*yellow*). Secondary structure prediction as in Fig. 2. This domain is longer than that proposed by Mouratou et al.; their coordinates for selected sequences are BIG1_Homs (1372-1489), BIG_Drom (1233-1350), SEC7_Sacc (1525-1704), BIG4_Arat (1278-1410), and hence all are 55 amino acids shorter than the domain proposed here. Coordinates in Mouratou et al. for GBF/Gea sequences are: GBF1_Homs (1532-1644), GBF1_Drom (1475-1666), GEA2_Sacc (1277-1433), GNOM_Arat (1235-1349), making the HDS3 domain defined here 50–80 amino acids longer than the one originally proposed

```
                                    1        10        20          30          40          50        60
GBF1_Homs/1532-1721  ADSRTLWAHCWCPLLQGIACLCCDARRQVRMQALTYLQ.RALLVHDLQ.KLDALEWESCFNKVLFPLLTKL
GBF1_Pant/1532-1721  ADSRTLWARCWCPLLQGIACLCCDARRQVRMQALTYLQ.RALLVHDLQ.KLDALEWESCFNKVLFPLLTKL
GBF1_Musm/1534-1723  ADSRTLWAHCWCPLLQGIACLCCDARRQVRMQALTYLQ.RALLVHDLQ.KLDALEWESCFNKVLFPLLTKL
GBF1_Ratn/1535-1724  ADSRTLWAHCWCPLLQGIACLCCDARRQVRMQALTYLQ.RALLVHDLQ.KLDALEWESCFNKVLFPLLTKL
GBF1_Mond/1531-1720  ADSRTLWAHCWCPLLQGIAWLCCDARRQVRMQALTYLQ.RALLVHDLQ.KLDALEWESCFNKVLFPLLTKL
GBF1_Canf/1545-1734  ADSRTLWAHCWCPLLQGIACLCCDARRQVRMQALTYLQ.RALLVHDLQ.KLDALEWESCFNKVLFPLLTKL
GBF1_Equc/1531-1720  ADSRTLWAHCWCPLLQGIACLCCDARRQVRMQALTYLQ.RALLVHDLQ.KLDALEWESCFNKVLFPLLTKL
GBF1_Drom/1475-1666  PQSAALWSPGWCPLLQGIARLAMDRRREVRTHAISCLQQRALLVHDLQ.TLSGTEWCSCFHQVLFPLLNEL
GBF1_Anog/1513-1701  PQCSSLWSQGWCPLLQGIARLATDQRRQVRTSAITCLQ.RALLVQDLQ.TLTGLEWAGCFKQVLFPLLQEL
GBF1_Tric/1375-1560  AKETSLWTQGWCPLLQGIARLCCDVRREIRMSAITYLQ.RALLVHDLQ.TLTGPEWEACFHRVLFPLLAHL
GBF1_Nasv/1321-1503  IATASLWPQAWRPLLQGIARLCCDSRRAVRAAAITSLQ.STLLAHDLS.QLSAVEWSQCLEQVLFPLLAQL
GEA1_Sacc/1271-1408  RSNSALNKNEIIAAIQALAHQCLNPCDELGMQALQALE.NILLSRASQLRTEKVAVDNLLETGLLPIFE..
GEA2_Sacc/1277-1431  EDNNDLRKNEIFAIIQALAHQCINPCKQISEFAVVTLE.QTLINKIEIPTNEMESVEELIEGGLLPLLN..
GEA_Klul/1230-1387   NTKPTVSKSEIIAIIQALSHQCLNPCFQLRSYALSSLE.NLLLKLLEPFVDNVISVIALIEMGLYPLTN..
GEA_Cang/1316-1454   NFPDFYTNEVIIAIVQALAHQCTNPCEQLRSYAVSSLQ.DSLIERIQIPSDQVQSLEQILEQGILPIL...
GEA_Schp/1250-1444   ESSEHFQDTRWKIIYDHVCKLCLSRSRSLRAASLSCLQ.RIVVEQLDQP.HEVSYTMALFLVLLPTMENM
GNL1_Arat/1227-1422  MKLSEDIGKMWLKLVKNLKKVCLDQRDEVRNHAISMLQ.RAIAGADGI.MLPQPLWFQCFDSAVFILLDDV
GNL2_Arat/1177-1372  LRGVNFVHHLFLKLSEAFRKTTLARREEIRNRAVTSLE.KSFTMGHEDLGFTPSGCIYCIDHVIFPTIDDL
GNOM_Arat/1235-1428  GKMSQDIGEMWLRLVQGLRKVCLDQREDVRNHALQSLQ.KCLGGVDGI.NLAHSMWSQCFDKVIFTVLDDL
GBF_Orys/1242-1436   DKVLEAIREMWLKLLQALKKLSLDQREEVRNHALTSLQ.RCLTATEGV.CLQSSTWSHAFDLVIFALLDDL
GBF_Vitv/1228-1422   SKMSQDIGEMWLRLVQGLRKVCLDQREEVRNHALISLQ.RCLSGVEGF.QLPHSLWLQCFDMVIFTMLDDL

                          70          80          90          100         110         120
GBF1_Homs/1532-1721  LE........NISPADVGGMEETRM.RASTLLSKVFLQHLSPLLSLSTFAALWLTILDFMDKYMHA....G
GBF1_Pant/1532-1721  LE........NISPADVGGMEETRM.RASTLLSKVFLQHLSPLLSLSTFAALWLTILDFMDKYMHA....G
GBF1_Musm/1534-1723  LE........NISPADVGGMEETRM.RASTLLSKVFLQHLSPLLSLSTFAALWLTILDFMDKYMHA....G
GBF1_Ratn/1535-1724  LE........NISPADVGGMEETRM.RASTLLSKVFLQHLSPLLSLSTFAALWLTILDFMDKYMHA....G
GBF1_Mond/1531-1720  LE........NISPADVGGMEETRM.RASTLLSKVFLQHLSPLLSLPTFAALWLTILDFMDKYMHA....G
GBF1_Canf/1545-1734  LE........NISPADVGGMEETRM.RASTLLSKVFLQHLSPLLSLSTFAALWLTILDFMDKYMHA....G
GBF1_Equc/1531-1720  LE........NISPADVGGMEETRM.RASTLLSKVFLQHLSPLLSLSTFAALWLTILDFMDKYMHA....G
GBF1_Drom/1475-1666  LPES....NAAGQLDAALLEESRI.RTATIMSKVFLQHLTTLIELNAFNELWLDILDYIERFMKV....G
GBF1_Anog/1513-1701  LQEK.....ATSPVEVSLLEESRI.RTATIMSKVFLHHLTPLIALPNFQELWIEILDYFERFMTA....G
GBF1_Tric/1375-1560  LS........NIDPKDPLAMEETRM.RAATVLSKVFLHHLTPLLSLPTFSNLWLIILDFIDKYMHA....D
GBF1_Nasv/1321-1503  LG........PIAANDPLAVEETRV.RAAMLLSKVFLHHLNPLLTLPGFLPLWLTVLDLLRSYMHA....D
GEA1_Sacc/1271-1408  .........LDEIQDVKMKRITSI.L..SVLSKIFLGQL..VEG.VTSNETFLRVLNVFNKYVD.....
GEA2_Sacc/1277-1431  .........SSETQE..DQKILISSILTIISNVYLHYLK.LG..KTSNETFLKILSIFNKFVE.....
GEA_Klul/1230-1387   .........EVDA.DS..RKAVPLQNFLSLLSKLYHYLS.LE..KTDNETYLKVLSIFNKHVD.....
GEA_Cang/1316-1454   .........LPEG.QN..ANSQHMKEVFPMITKVYFHYLK.KG..STTNDTFLRVLNVMFNSCVE.....
GEA_Schp/1250-1444   ITVL....TEKPEHKIFGLAQA.QMFNIICKTVLIDMNVLSAQ...KEMLHTLWLKLMDVAIKLSSIHG.S.
GNL1_Arat/1227-1422  LTFS....IENSRKTLKKTVEETLV.LATKLMSKAFLQSLQDISQQPSFCRLWVGVLNRLETYMSTEFRGK
GNL2_Arat/1177-1372  HEKLLDYSRRENAEREMRSMEGTLK.IAMKVLMNVFVYLEQIVESAEFRTFWLGVLRRMDTCMKADLGEY
GNOM_Arat/1235-1428  LEIA....A..GSQKDYRNMEGTLL.LAMKLLSKVFLQQLQELSQLSTFCKLWLGVLTRMEKYMKVKVRGK
GBF_Orys/1242-1436   LEIS....Q.NHSQKDYRNMEGSLV.LAMKLVAKVLQLLPDLFGLSSFCKLWLGVLSRMEKYIKIKVRGK
GBF_Vitv/1228-1422   LDIA....Q.GHSQKDYRNMEGTLS.LAMKLLSKVFLQLLNDLAQLTTFCKLWLGVLSRMEKYMKVKVKGK

                          130         140         150         160         170         180         190
GBF1_Homs/1532-1721  SSDLLSEAIPESLKNMLLVMDTAEIFHSADARGG......GPSALWEITWERIDCFLPHLRDELFKQTV
GBF1_Pant/1532-1721  SSDLLSEAIPESLKNMLLVMDTAEIFHSADARGG......GPSALWEITWERIDCFLPHLRDELFKQTV
GBF1_Musm/1534-1723  SSDLLSEAIPESLKNMLLVMDTAEIFHSADARGG......GPSALWEITWERIDCFLPHLRDELFKQTV
GBF1_Ratn/1535-1724  SSDLLSEAVPESLKNMLLVMDTAEIFHSADARGG......SPSALWEITWERIDCFLPHLRDELFKQTV
GBF1_Mond/1531-1720  SSDLLSEAIPESLKNMLLVMDTAEIFHNADTRGS......GSSALWEITWERIDCFLPHLRDELFKQTV
GBF1_Canf/1545-1734  SSDLLSEAIPESLKNMLLVMDTAEIFHNADARGG......SPSALWEITWERIDCFLPHLRDELFKQTV
GBF1_Equc/1531-1720  SSDLLSEAIPESLKNMLLVMDTAEIFHSADARGG......SPSALWEITWERIDCFLPHLRDELFKQTV
GBF1_Drom/1475-1666  .SDTLSEQMQEILKNMLLVMHSVRVFHNQD..GS......LQQALWELTWRRIGEFLPNLKEELFHDEG
GBF1_Anog/1513-1701  .SDMLYEAVLESLKNMLLVMHSVCVFHNTD..GV......THSMLWDVTWQRISGFLPNLKDELFKHEG
GBF1_Tric/1375-1560  KSDLLAEAIPESLKNMLLVMDSAKVFDGPD........GKSPLWAATWDRINKFLPGMKEELFREQQ
GBF1_Nasv/1321-1503  NSENLYEAIPESLKNMLLVMASAGVLQP...........ESYLWTPTWRAIDTFLPNLKAELFPEPP
GEA1_Sacc/1271-1408  ..DPTVERQLQELIISKREIEKE.........
GEA2_Sacc/1277-1431  ..DSDIEKKLQQLILDKKSIEKGNGSSSHGSAHEQTPESNDV....
GEA_Klul/1230-1387   ..DGKVEEQLQDMITKKREIQKGGTTPSTDAESVPAEPTPVSK....
GEA_Cang/1316-1454   ..DPEIENQLQQFIIMKKEIEAEST.......
GEA_Schp/1250-1444   ..ESMAE.VMESIKNVFMILHGAGALAGPT.IEVDPEIPDHLIKTWNTTWNNLFIYYPELSNDLNINN.
GNL1_Arat/1227-1422  RSEKVNELIPELLKNTLLVMKATGVLLPGDDIGS.......DSFWQLTWLHVNKISPSLQSEVFPQEE
GNL2_Arat/1177-1372  GDNKLQEVVPELLTTMIGTMKEKEILVQKE.........DDDLWEITYIQIQWIAPALKDELFPD..
GNOM_Arat/1235-1428  KSDKLQESVPELLKNILLVMKTKGVLLQRSALGG.......DSLWELTWLHVNNIAPSMRLELFPDQE
GBF_Orys/1242-1436   RSDKLQELIPELLKNILIAMKNRGILAKRSTIGG.......DSLWELTWLHANNISTSLQSDVFPSQE
GBF_Vitv/1228-1422   RSEKLPELVPELLKNTLLVMKTRGVLVQRSALGG.......DSLWELTWLHVNNIAPTLQSEVFPDQV
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Fig. 9 Conserved residues among members of the GBF/Gea subfamily within the region including the HDS3 domain. Multiple sequence alignment showing conserved residues (*blue*) and invariant residues (*red*). Only sequences from animals, fungi, and plants are included. Secondary structure prediction of alpha-helical regions is shown above alignment in *blue*

identified interacting proteins of the GBF/Gea and the BIG/Sec7 Arf1 GEFs (Table 3). It is important to point out that many of these interacting partners have been identified by yeast two-hybrid screens or by co-immunoprecipitation of proteins from cells, which demonstrate a physical association, but not a direct protein–protein interaction. Further studies are required to determine whether these interactions are direct or via a bridging protein. Many of the interactions described in Table 3 have been previously reviewed (Cox et al. 2004; Shin and Nakayama 2004; Mouratou et al.

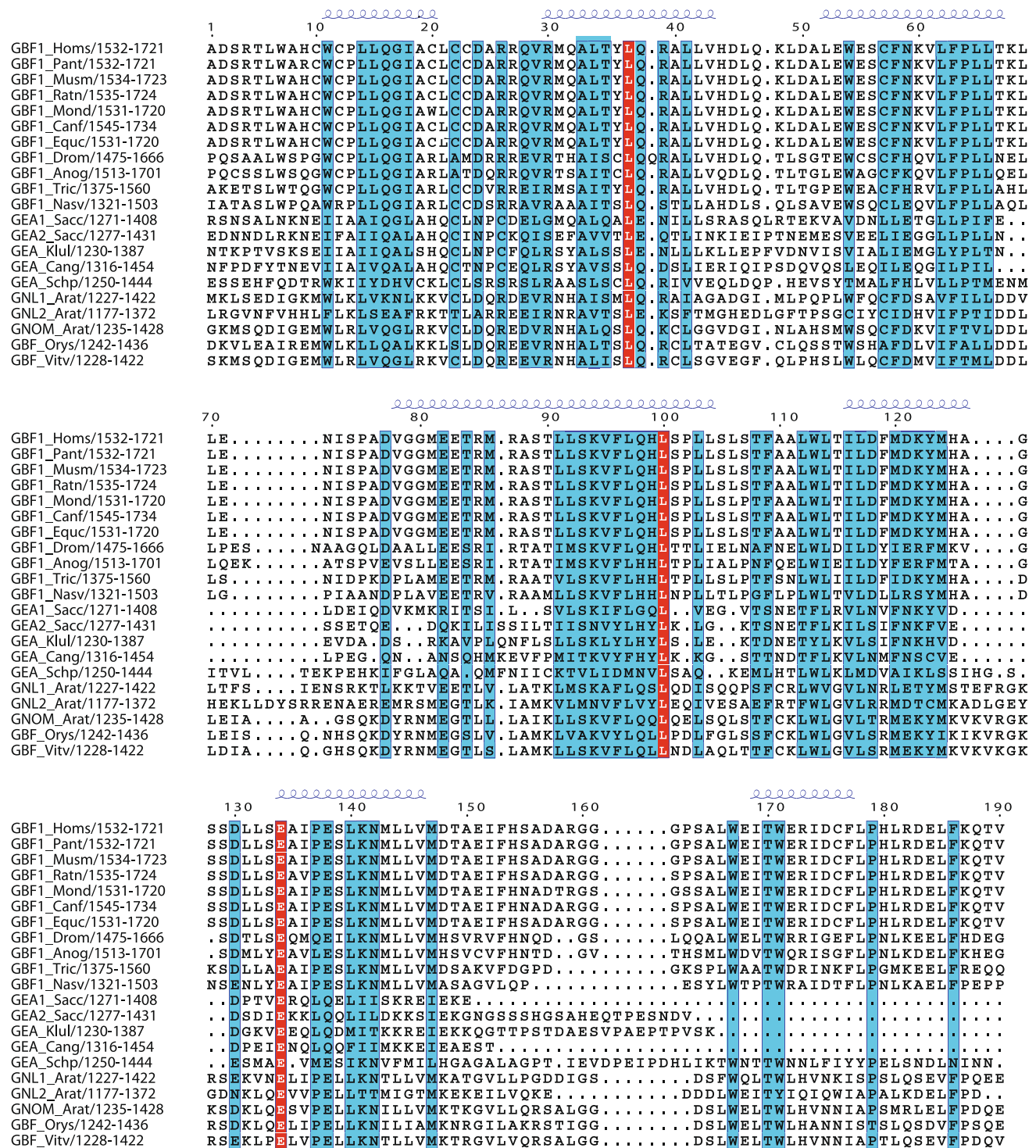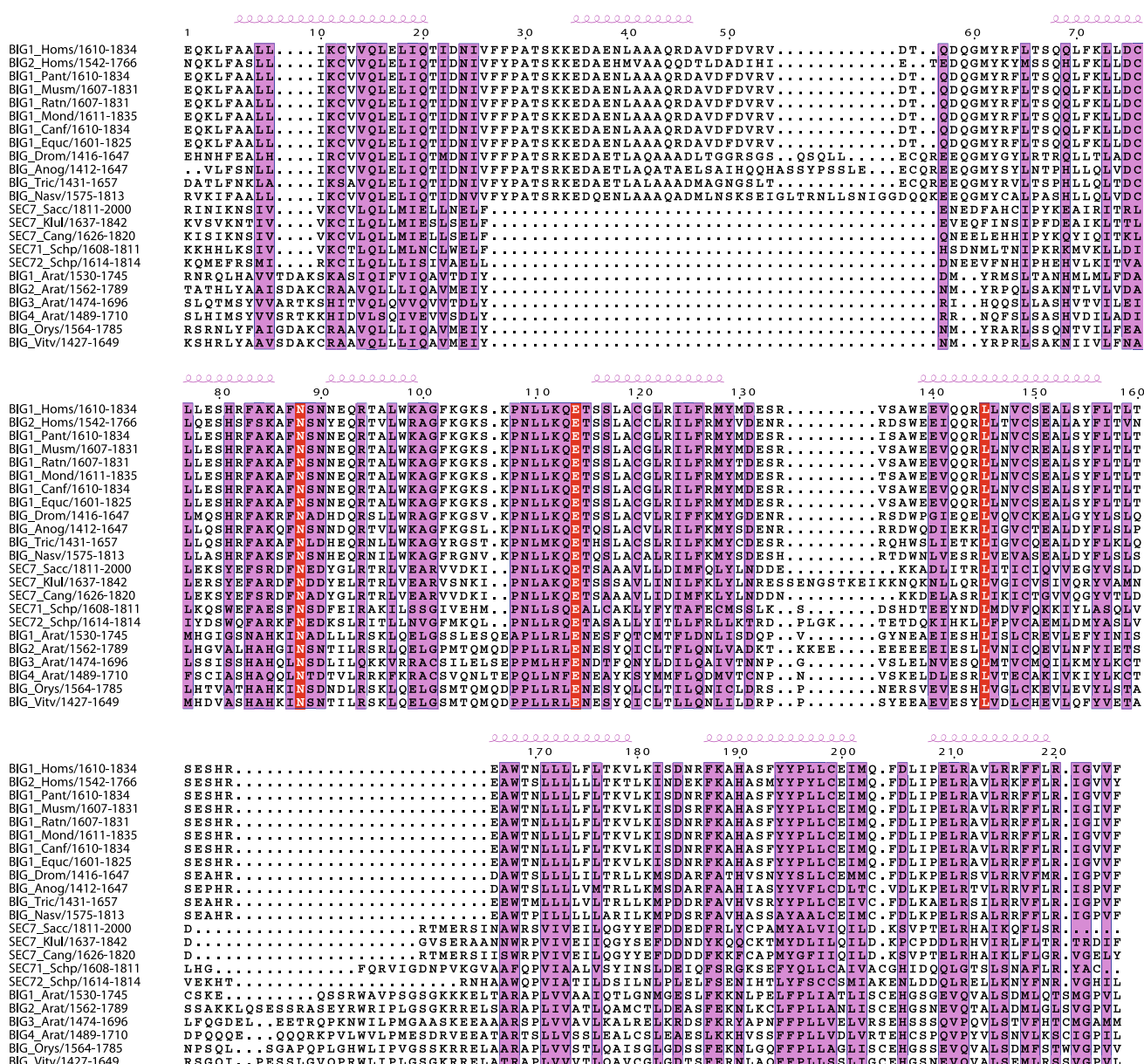**Fig. 10** Conserved residues among members of the BIG/Sec7 subfamily within the C-terminal region including the HDS4 domain. Multiple sequence alignment showing conserved residues (*pink*) and invariant residues (*red*). Secondary structure prediction of alpha-helical regions is shown above alignment in *pink*

2005; Casanova 2007; Anders and Jurgens 2008), so we will focus here on more recent results.

The Arf1 GEF interactions for which a direct protein–protein interaction has been demonstrated are listed in the first part of Table 3. For BIG1, these include the exocyst subunit Exo70 (Xu et al. 2005), the Myosin IXb molecular motor (Saeki et al. 2005), and the BIG1 protein itself (Ramaen et al. 2007). The interactions with Exo70 and Myosin IXb suggest a role for BIG1 in targeting secretory vesicles to the PM. The BIG1 DCB domain has been demonstrated directly to form a dimer through purification of this domain from *E. coli* and gel filtration analysis (Ramaen

et al. 2007). This data confirmed the initial observations for the *Arabidopsis thaliana* GNOM Arf1 GEF that the DCB domain is involved in dimerization (Grebe et al. 2000).

For mammalian GBF1, two proteins have been shown to interact directly, the small G protein Rab1 (Monetta et al. 2007) and the COPI coat subunit gamma-COP (Deng et al. 2009). Rab1 plays an essential role in trafficking between the ER and *cis*-Golgi (Behnia and Munro 2005). Like the Arf small G proteins, Rabs cycle between GDP- and GTP-bound forms, and target effector proteins to membranes when in their active GTP-bound conformation. Rab proteins are primarily involved in membrane fusion, with

Table 3 Interactions between the Arf1 GEFs and protein partners

Arf GEF	Species	Interacting protein	Interaction domain	Technique showing direct interaction in vitro	References
Direct protein–protein interactions					
BIG/Sec7 subfamily					
BIG1	*Hs*	Myosin IXb molecular motor	1,305–1,849	Epitope-tagged proteins purified from baculovirus, immunoaffinity column	Saeki et al. (2005)
		Exo70 exocyst complex component	1–643	CoIP of in vitro-translated, epitope-tagged proteins	Xu et al. (2005)
		BIG1	DCB	Purification from *E. coli*, gel filtration	Ramaen et al. (2007)
BIG2	*Rn*	β-subunit of GABA receptor gamma-aminobutyric acid type A receptor	1,739–1849	GST- and His-tagged proteins purified from *E. coli*, gel overlay assay	Charych et al. (2004)
Gea/GBF subfamily					
GBF1	*Hs*	Rab1b small G protein	1–380	GST- and His-tagged proteins purified from *E. coli*, GST column	Monetta et al. (2007)
		γ COP COPI coat subunit	nd	GST- and His-tagged proteins purified from *E. coli*, GST column	Deng et al. (2009), Golinelli-Cohen and Jackson, unpublished data
Gea1/2p	*Sc*	Drs2p aminophospholipid translocase	Sec7	Purification from *E. coli*, transcription–translation in vitro, immunoaffinity column	Chantalat et al. (2004)
		Sec21p COPI coat subunit	N-Sec7	GST- and His-tagged proteins purified from *E. coli*, GST column	Deng et al. (2009)

Arf GEF	Species	Interacting protein	Interaction domain	Technique showing physical association	References
Physical associations (possibly direct but not demonstrated)					
BIG/Sec7 subfamily					
BIG1/2	*Hs*	FKBP13 FK506-binding protein	1–331	2-hybrid, coIP	Padilla et al. (2003)
		PP1-γ protein phosphatase	nd	Kinase-anchoring proteins sequence, phosphorylation	Kuroda et al. (2007)
	Hs	3CD poliovirus protein	nd	Translation of 3CD in cell extracts, Western analysis of membrane fractions	Belov et al. (2007b)
BIG1	*Hs*	Fibrillarin part of snRNP particle U3 snoRNA	nd	CoIP	Padilla et al. (2008)
		Integrin β1	nd	Necessary for correct glycosylation of integrin β1	Shen et al. (2007)
		KIF21A kinesin motor protein	885–1,849	CoIP, colocalization	Shen et al. (2008)
		La RNA binding protein	nd	CoIP	Padilla et al. (2008)
		Nucleolin synthesis and maturation of ribosomes	nd	CoIP	Padilla et al. (2004, 2008)
		Nucleoporin 62 import of protein to the nucleus	nd	CoIP	Padilla et al. (2004)
BIG2	*Hs*	AMY-1 c-Myc interacting protein, AKAP-binding	DCB	CoIP, siRNA	Ishizaki et al. (2006)
		R subunits of PKA regulatory subunits of type I protein kinase	27–48 (RIα, RIβ), 284–301 (RIIα, RIIβ), 517–538 (RIα, RIIα, RIIβ)	2-hybrid, coIP	Li et al. (2003)
		TNFR1 tumor necrosis factor receptor	nd	Effect of siRNA/BFA on the release of TNFR1, colocalisation, co-IP	Islam et al. (2007, 2008)

Table 3 continued

Arf GEF	Species	Interacting protein	Interaction domain	Technique showing physical association	References
Sec7p	*Sc*	COPI coat complex	493–1,196	GST-pulldown using purified GST-Sec7 fragments and yeast cytosol	Deitz et al. (2000)
		COPII coat complex	1,196–2,010	GST-pulldown using purified GST-Sec7 fragments and yeast cytosol	Deitz et al. (2000)
Gea/GBF subfamily					
GBF1	*Hs*	AMP-activated protein kinase (AMPK)	nd	Phospho-akt-substrate antibody	Miyamoto et al. (2008)
		GBF1	DCB and HUS	2-hybrid, coIP	Ramaen et al. (2007)
		GGA adaptor protein for clathrin	DCB–HUS	Colocalization, 2-hybrid, coIP, siRNA	Lefrancois and McCormick (2007)
		p115 tether	1,752–1,859	2-hybrid, coIP, GST-pulldown using cytosol, colocalization	Garcia-Mata and Sztul (2003)
		3A protein polio and coxsackieviruses protein	1–566	2-hybrid, coIP, colocalization	Wessels et al. (2006), Belov et al. (2007a)
GNOM	*At*	CYP5 peptidyl-prolyl transisomerase	1–146	2-hybrid, GST-pulldown using GST-CYP5 and Arabidopsis protein extracts	Grebe et al. (2000)
		GNOM	DCB, GNOMΔDCB	2-hybrid, in vivo complementation	Grebe et al. (2000), Anders et al. (2008)
Gea1/2p	*Sc*	Gmhlp Golgi integral membrane protein	HDS1–3	2-hybrid, co-IP, colocalization	Chantalat et al. (2003)
		Scp160p KH-domain RNA binding protein		2-hybrid, co-purification	Peyroche and Jackson (2001)

Hs Homo sapiens, *Rn Rattus norvegicus*, *Sc Saccharomyces cerevisiae*, *At Arabidopsis thaliana*, *nd* not determined

roles in regulation of tethering complexes and the pairing of SNAREs which directly mediate fusion (Behnia and Munro 2005). GBF1 was shown to be an effector for Rab1 in vitro, and active Rab1 in cells was shown to increase the association of both GBF1 and COPI to membranes (Monetta et al. 2007). Interestingly, another Rab1 effector, the tethering protein p115, has also been shown to interact with GBF1 (Garcia-Mata and Sztul 2003). p115 not only acts to tether membranes in ER–Golgi trafficking, but also interacts directly with and promotes pairing of the ER–Golgi SNARE proteins, which are required for membrane fusion (Short et al. 2005). A precise molecular explanation for the Rab1–GBF1–p115 series of interactions has not been demonstrated, but suggests a role for GBF1 in coordination of vesicle budding and fusion processes.

The interaction between GBF1 and the gamma-COP subunit of the COPI coat complex is conserved from yeast to humans. This interaction is a direct protein–protein interaction in both systems (Deng et al. 2009; MPG-C and CLJ, unpublished data). Studies from several laboratories have indicated that GBF1 is involved specifically in recruitment of COPI to early Golgi membranes. Knockdown of GBF1 by RNAi inhibits COPI association with membranes with-

out affecting other coats such as AP-1 (Ishizaki et al. 2008; Manolea et al. 2008; Deng et al. 2009). In contrast, knockdown of the BIG1 and BIG2 proteins prevents AP-1 association with TGN membranes, without affecting COPI (Ishizaki et al. 2008; Manolea et al. 2008). A model explaining this specificity has been proposed, whereby COPI interacts with the Arf1 GEF GBF1 (Gea1p and Gea2p in yeast) prior to nucleotide exchange on Arf1, so that the effector COPI is in place at the time Arf1-GTP is formed (Deng et al. 2009). This model offers an explanation for the observation that a single Arf protein, Arf1, can recruit at least five different coat complexes, including COPI, AP1/clathrin, GGA/clathrin, AP3, and AP4, to different sites within cells (Bonifacino and Lippincott-Schwartz 2003). Interestingly, all these coat complexes share sequence homology and are structurally similar (Bonifacino and Lippincott-Schwartz 2003; McMahon and Mills 2004), and function in a specific trafficking pathway (Bonifacino and Lippincott-Schwartz 2003; McMahon and Mills 2004). This is also true of the large Arf1 GEFs, whose function is required to recruit these coat complexes to membranes. One of the regions of gamma-COP that interacts with the Arf1 GEFs is the appendage domain

(Deng et al. 2009), a region that is structurally conserved in all five coats recruited to membranes by Arf1-GTP (McMahon and Mills 2004). These results suggest the possibility that the different Arf1 GEFs within these two subfamilies regulate coat assembly via a common mechanism involving GEF-coat interactions.

An interaction between GBF1 and GGA coat complexes has been proposed, although it has not been determined if this interaction is direct (Lefrancois and McCormick 2007) (Table 3). GBF1 functions and localizes to the *cis*-Golgi, whereas the GGA proteins function and localize primarily to the *trans*-Golgi and TGN (Hirst et al. 2001; Puertollano et al. 2001; Mattera et al. 2003; Nakayama and Wakatsuki 2003; Puertollano et al. 2003). Severe perturbations of the early Golgi caused by interfering with GBF1 function also affect late-Golgi functions, where the GGA proteins act. Hence, the results of this study are likely due to a more indirect mechanism than by direct interaction of GBF1 and the GGAs.

An important role for the Arf1 GEFs in cell signaling has emerged with the identification of BIG1 and BIG2 as A-kinase anchoring proteins or AKAPs. AKAPs are PKA regulatory subunit binding proteins that act as scaffolds to compartmentalize signaling cascades. This function is accomplished through formation of multi-protein complexes that spatially restrict the PKA-cAMP signaling cascade at specific membrane sites within cells. BIG1 and BIG2 both contain AKAP domains that mediate binding of the regulatory subunit RIIβ of protein kinase A (PKA) (Figs. 2, 3), and BIG1 accumulates in nuclei upon increase in cAMP levels in cells (Li et al. 2003; Padilla et al. 2004; Citterio et al. 2006). Both BIG1 and BIG2 immunoprecipitated from HepG2 cells can be phosphorylated by recombinant PKA, and this results in a decrease in GEF activity (Kuroda et al. 2007). One PKA phosphorylation site in BIG1 has been identified, at the C-terminus of the Sec7 domain (Li et al. 2003) (Fig. 4). Furthermore, endogenous protein phosphatase 1-γ (PP1-γ) was found in a complex with BIG1 or BIG2 in microsomal fractions, indicating that a phosphatase of this signaling pathway is physically associated with BIG1 and BIG2 GEFs (Kuroda et al. 2007). A recent study from this group has shown that another regulatory enzyme in this cascade, phosphodiesterase PDE3A, is functionally associated with BIG1 and BIG2 (Puxeddu et al. 2009). PDE enzymes break down cAMP to terminate cAMP signaling, reversing its effects on BIG1 and BIG2. Puxeddu et al. showed that PDE3A (but not PDE4) co-immunoprecipitates with both BIG1 and BIG2, and that knockdown of PDE3A led to a partial redistribution of BIG1 and BIG2 from Golgi membranes to cytosol. In addition to the cAMP-PKA regulatory proteins themselves, an AKAP-binding protein AMY-1 has also been found to associate with the RIIβ-binding (AKAP) domains of both

BIG1 and BIG2 (Ishizaki et al. 2006). In cells, AMY-1 TGN localization is abrogated in cells depleted of BIG2, but not of BIG1, indicating that BIG2 is the physiological partner of AMY-1 at the TGN (Ishizaki et al. 2006).

What are the functional consequences of BIG1 and BIG2 involvement as AKAPs in cAMP-PKA signaling? For BIG1, phosphorylation by PKA results in its redistribution from the Golgi and cytoplasm to the nucleus (Citterio et al. 2006). The precise function of BIG1 in the nucleus is not known, but it involves interactions with nuclear proteins including nucleolin, fibrillarin and RNA-binding protein La, as well as the small nuclear RNA snoU3 (Padilla et al. 2008).

The type I tumor necrosis factor receptor (TNFR1) is one of two receptors for TNF that mediates its effects on inflammation, apoptosis, and the innate immune response. TNFR1 can be released from cells by incorporation into exosome-like vesicles which are shed from the surface of cells (Hawari et al. 2004). BIG2 has been shown to play a role in regulating this process by two mechanisms, one involving its GEF activity, the other through its AKAP domains. Activation of Arf1 and Arf3 by BIG2 is required for extracellular release of TNFR1 in exosome-like vesicles, and involves a physical association between BIG2 and TNRF1 (Islam et al. 2007). In addition, cAMP-dependent protein kinase A signaling induces TNFR1 exosome-like vesicle release through binding of PKA regulatory subunit RIIβ to AKAP domains of BIG2 (Islam et al. 2008).

Arf1 GEFs and human disease

Mutations in the BIG2 Arf1 GEF have been linked to autosomal recessive periventricular heterotopia (ARPH), a disorder of neuronal migration that leads to severe malformation of the cerebral cortex (microcephaly) and severe developmental delay (Sheen et al. 2004). Two BIG2 disease alleles have been identified, including a frameshift mutation that results in truncation of the majority of the protein (Sheen et al. 2004). The disease symptoms are a result of the failure of a specific class of neurons to migrate from their point of origin in the lateral ventricular proliferative zone to the cerebral cortex (Sheen et al. 2004; Ferland et al. 2009). This defect arises from a defect in vesicular trafficking that alters the adhesion properties of these neurons (Ferland et al. 2009). Interestingly, mutations in other trafficking proteins can give rise to periventricular heterotopia in humans or in model systems, indicating that trafficking defects are likely the root cause of this disease (Ferland et al. 2009). Hence, the Arf1 GEFs not only serve essential roles in fundamental cell biological processes, but also play an

important role in human development and neuronal function (Sheen et al. 2004).

The Arf1 GEFs have been shown to act as host factors for pathogens mediating human disease. Replication of several viruses has been shown to require GBF1, including poliovirus, coxsackievirus, and coronavirus. Poliovirus and coxsackievirus are enteroviruses belonging to the *Picornaviridae* family of positive strand non-enveloped RNA viruses. Despite the fact that enteroviruses are not enclosed by a membrane, their replication never-the-less depends completely on host cell membranes. Upon infection, enteroviruses cause a massive reorganization of the intracellular membranes of their host, including ER and Golgi membranes (Bienz et al. 1983; Salonen et al. 2005). Replication of poliovirus and coxsackievirus is completely inhibited by BFA, and it has been shown that GBF1 is the major target of this drug in poliovirus replication (Belov et al. 2007a; Belov et al. 2008). The enteroviral 3A protein has also been shown to block secretion in host cells, which inhibits the innate immune response of the host (Doedens et al. 1997; Wessels et al. 2006). This block in secretion also occurs via GBF1, through physical association of 3A with GBF1 which inhibits its function on Golgi membranes in the host cell (Wessels et al. 2006; Wessels et al. 2007). Hence, polio and coxsackieviruses subvert the host membrane by blocking GBF1 activity on host cell Golgi membranes, reorganizing these membranes, then activating GBF1 function on Arf1 for viral replication on these virally induced membrane structures.

Although a major role for GBF1 in enteroviral replication has been demonstrated, there is evidence that the BIG1 and the BIG2 Arf1 GEFs are also involved. Interestingly, different viral proteins have been shown to recruit different coats to virally reorganized membranes. In an in vitro system, it has been shown that the 3A protein recruits GBF1 to membranes (Belov et al. 2008), whereas the viral 3CD protein specifically recruits BIG1 and BIG2 (Belov et al. 2007b). Furthermore, 3A recruitment of GBF1 results in specific binding of COPI to membranes (Belov et al. 2008), whereas, 3CD recruits the GGA3 coat to membranes (Belov et al. 2007b). These observations support an intimate coupling between recruitment of specific GEFs with specific coats to membranes, and provide another line of evidence supporting a connection between GBF1 and COPI, and also a connection between BIG1/BIG2 and the late-Golgi coat GGA3.

In addition to polio and coxsackieviruses, the RNA replication of a number of positive-strand viruses is BFA-sensitive (Gazina et al. 2002; Molina et al. 2007; Tai et al. 2009). Among these is Hepatitis C virus (HCV), which has been shown recently to depend also on the Arf1 effectors COPI and phosphatidylinositol-4-kinase for its replication (Tai et al. 2009). These results strongly suggest that GBF1

is a required host factor for HCV replication. BFA also inhibits replication of other families of viruses, including the enveloped positive-strand RNA coronaviruses (Verheije et al. 2008). For one member of the coronaviruses, mouse hepatitis coronavirus, GBF1 and Arf1 have been demonstrated to be essential host factors for its RNA replication (Verheije et al. 2008).

Given the important roles of the Arf1 GEFs of the GBF/Gea and BIG/Sec7 subfamilies in human disease, development of drugs that specifically target these proteins is of medical interest. As mentioned above, BFA is a natural substance that is a potent and specific inhibitor of members of both of these subfamilies. Recently, a chemical (Golgicide A) which specifically inhibits GBF1, but not BIG1 or BIG2, has been described (Saenz et al. 2009). Golgicide A was identified in a screen for inhibitors of the cytotoxicity of Shiga toxin, a protein produced by the pathogenic bacterium *Shigella dysenteriae* (Saenz et al. 2009). This discovery, in addition to providing an important inhibitor of GBF1, demonstrates a crucial role for this Arf1 GEF as a host factor in *S. dysenteriae* infection.

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